Evaluation of Malthus 128H microbiological growth analyser for detecting significant bacteriuria

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SUMMARY In a pilot study a prototype Malthus 128H microbiological growth analyser was used to study the growth of six commonly encountered urinary pathogens in a range of five different laboratory media. No single medium had been able to show acceptable conductance changes for all bacteria within five hours. Subsequently, the Malthus system, with fastidious anaerobe broth as the culture medium, was used to screen 500 consecutive clinical specimens of urine for significant bacteriuria. After two and a half hours the Malthus system detected 32 out of the 40 true positives and eight of the 44 contaminated urines. Increasing the detection time to five hours permitted the recognition of 39 true positives and 37 contaminated urines.

Conventional methods for diagnosing urinary tract infection entail the quantitative or semi quantitative culture of specimens of urine on solid culture media. Results are read after overnight incubation, and a pure growth of a single bacterial species in numbers $>10^5$ organisms/ml is accepted as indicative of infection.¹ Diagnostic laboratories investigate thousands of urine specimens each year but of these only a small proportion (about 10%) prove to be infected. Large amounts of both staff time and culture medium are consequently expended on specimens that eventually prove to be sterile.

Many screening methods have been advocated for use in detecting bacteria in urine. These include measurements of bacterial adenosine triphosphate by luciferase,² measurement of heat generated by metabolising organisms,³ changes in electrical impedance,⁴ and three automated photometric systems—Autobac (General Diagnostics), Auto Microbic System (Vitek Systems Inc), and MS-2 (Abbott Laboratories)—which detect growth by changes in light transmission⁵ and also particle counting.⁶ The Malthus system measures the conductance between two electrodes immersed in media. Growth of bacteria in the culture is indicated by a change in the electrical impedance of the growth medium that has been shown to be proportional to changes in the conductance component of the impedance.⁷ Brown and Warner examined the possibility of using the Malthus system to detect bac-

teriuria.⁸ We report the results of a further investigation in which we evaluated the Malthus system in 500 urine specimens in comparison with our standard laboratory method.

Material and methods

MALTHUS SYSTEM

The machine used was a prototype Malthus 128H microbiological growth analyser⁹ controlled by a Motorola exorset microcomputer with peripheral units of a matrix printer (ET-5200) and a digital plotter (H1-Plot). The thermostatically controlled water bath contained 128 growth cells. The computer was programmed for a total scan time of five hours and each of the cells was scanned with a 10k Hz signal every six minutes. Data from each cell were available on demand either on a visual display unit or on the digital plotter as a graph which indicated the change of conductance over time. Preliminary work had shown that a suitable detection routine entailed three successive increases of conductance of 4 μ S.

MEDIA

Five media, Mueller-Hinton, brain-heart infusion, Todd-Hewitt, Isosensitest (Oxoid), and fastidious anaerobe broth (FAB, Lab M) were tested for their ability to support detectable growth by the Malthus system using one strain each of the following commonly encountered urinary pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, group B streptococcus, *Staphylococcus*

aureus, and *Staphylococcus saprophyticus*. All bacterial strains had been taken from clinical specimens of infected urine. As the accepted criteria for urinary tract infection is $>10^5$ organisms/ml suspensions containing this amount were used to inoculate the system, 0.02 ml being added to each cell. In preliminary experiments, 10 ml growth cells had been used. Using these, it was found that only *E coli* gave an adequate response. A change to the alternative, smaller 2 ml growth cells produced a pronounced improvement in time taken for detection of all the test organisms.

URINE SAMPLES

Five hundred consecutive urine specimens were cultured on CLED agar using a standard 0.01 ml nichrome wire loop.¹⁰ A pure growth in excess of 10^5 organisms/ml was regarded as confirmation of infection and all organisms were identified by standard techniques.^{11,12} The growth cells of the Malthus system were inoculated by "dipping" the ceramic strip containing the platinum electrodes into the specimen. The ceramic strip was then replaced into the cell and connected to the apparatus by means of push on connectors. Previous work had determined that 0.02 ml of sample was consistently transferred from specimen to cell using this "dip" technique. Each specimen was incubated for up to five hours, with every cell being subcultured on to a Columbia blood and CLED agar plate at the end of the incubation.

Results

Within five hours brain-heart infusion, Mueller-Hinton, and Isosensitest media were unable to sustain detectable growth of *Ps aeruginosa* and *Staph aureus*. Todd-Hewitt and fastidious anaerobe broth required the shortest time to detect the six test organisms. Using these two media, *E coli*, *S faecalis*, and group B streptococcus were detected within one and a half hours; *Staph aureus*, *Staph saprophyticus*, and *Ps aeruginosa* were detected in four hours. *Staph saprophyticus* could be detected within one and a half hours when urea was added to the medium at a final concentration of 1%. The addition of urea, however, prolonged the time needed to detect *Ps aeruginosa*.

After investigation into the most suitable media fastidious anaerobe broth (without urea) was the medium chosen to screen 500 consecutive clinical specimens of urine. Of the 500 urines, 40 yielded a significant ($>10^5$ organisms/ml) pure growth of bacteria by the laboratory's standard method: 37 *Enterobacteriaceae*; one *Ps aeruginosa*; one *S faecalis*; and one *Staph saprophyticus*. A further 44 specimens yielded a growth of mixed bacteria at levels of $>10^5$ organisms/ml. The table shows the cumulative totals. At two and a half hours 80% of the samples with significant bacteriuria were detected but so were 18% of the contaminated urines. By extending the detection time the number of contaminated urines detected increased greatly. Of the eight infections not detected by the Malthus system within two and a half hours, five were due to *E coli* (the bacterial growth having been inhibited by the presence of boric acid in the container), one *Ps aeruginosa*, one *S faecalis*, and one *Staph saprophyticus*.

Discussion

We evaluated the Malthus system for its ability to detect the growth of potential urinary pathogens in liquid media, and subsequently, in the examination of 500 samples of urine. No single medium was suitable for the detection of all six test organisms. If the variables for conductance change were adjusted to allow for greater sensitivity the growth of all test organisms was detected but the discrimination was reduced to such a degree that even uninoculated broth gave false positive results. Fastidious anaerobe broth seemed to be the best medium but performed badly in the detection of *Staph saprophyticus*. This problem was alleviated by the addition of 1% urea, but this retarded the detection of *Ps aeruginosa*. The addition of 1% urea was stopped because of this effect and also because, as it is heat labile, urea has to be added after the autoclaving of the growth cells, which increases the risk of contamination.

The original 10 ml cells were replaced by the smaller 2 ml growth cells, which enhanced the growth of all organisms. Enhanced growth could have been due to either a higher initial concentration of organisms or to an increased head space. For

Time required to detect organisms using Malthus system and conventional urine cultures

Conventional culture ($>10^5$ Organisms/ml)	Detection (hours)									
	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5
Pure growth (n = 40)	8	15	25	27	32	35	36	37	39	39
Mixed growth (n = 44)	1	1	3	8	8	14	16	22	35	37

routine clinical use of the system, it would be necessary to determine a cut off time that would distinguish between true positives—that is, patients with significant bacteriuria—and false positives—that is, contaminated specimens.

If this cut off time were set at two and a half hours, 80% of the true positives would be detected but so would 18% of the contaminated urines. To detect 100% of the true positives the cut off time would have to be increased to five hours as has been previously advocated⁸ and this would result in a positive result for most contaminated urines as the Malthus system cannot be expected to differentiate between pure and mixed cultures. It is worth noting also that many hospitals use boric acid as a preservative for urine specimens that may be delayed in transit. Boric acid effectively inhibits the multiplication of contaminants, but, unfortunately, it also delays the growth of true infecting organisms in the Malthus system, and thus leads to false negative results. The system in its present form, therefore, is not suitable for use when this preservative is used.

Microbiological growth analysers clearly offer interesting possibilities in the field of diagnostic microbiology. Their most obvious application at this time seems to be in the diagnosis of bacteraemia,¹³ in which the probability of a single infecting species is high and the likelihood of a contaminated specimen is comparatively low. In their present form we agree with the views of earlier work;⁸ their usefulness in screening for significant bacteriuria is limited. A particular difficulty is the lack of a suitable medium that allows the detectable growth of all urinary pathogens within a realistic time. The manufacturers are about to release a specialised Columbia broth, which has been optimised for conductance measurements with the Malthus system, and this, they claim, will detect all the test organisms. This medium, which was not available at the time of our evaluation, could greatly enhance the system's value.

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References

- ¹ Kass EH. Bacteriuria and the diagnosis of infection of the urinary tract. *Arch Intern Med* 1957;**100**:709–14.
- ² Mackett D, Kessock-Philip S, Bascomb S, Easmon CSF. Evaluation of the Lumac kit for the detection of bacteriuria by bioluminescence. *J Clin Pathol* 1982;**35**:107–10.
- ³ Beezer AE, Bettelheim KA, Newell RD, Stevens J. The diagnosis of bacteriuria by flow microcalorimetry, a preliminary investigation. *Science Tools* 1974;**21**:13–5.
- ⁴ Cady P, Dufour SW, Lawless P, Nunke B, Kraeger SJ. Impedimetric screening for bacteriuria. *J Clin Microbiol* 1978;**7**:273–8.
- ⁵ Pezzlo MT, Tan GL, Petersen EM, de la Maza LA. Screening of urine cultures by three automated systems. *J Clin Microbiol* 1982;**15**:468–74.
- ⁶ Dow CS, France AD, Khan MS, Johnson T. Particle size distribution analysis for the rapid detection of microbial infection of urine. *J Clin Pathol* 1979;**32**:386–90.
- ⁷ Richards JCS, Jason AC, Hobbs G, Gibson DM, Christie RH. Electronic measurement of bacterial growth. *J Phys (Sect E)* 1978;**2**:560–8.
- ⁸ Brown DJF, Warner M. Impedance and conductivity methods for detecting bacteriuria. In: Tilton RC, ed. *Rapid Methods and Automation in Microbiology*. Washington: American Society for Microbiology, 1982:171–5.
- ⁹ Baynes NC, Comrie J, Prain JH. Detection of bacterial growth by the Malthus conductance meter. *Med Lab Sci* 1983;**40**:149–58.
- ¹⁰ Mackey JP, Sandys GH. Diagnosis of Urinary Infections *Br Med J* 1966;**i**:1173.
- ¹¹ Cowan ST, Steel K. *Manual for the identification of Medical Bacteria*, 2nd ed. Cambridge: Cambridge University Press, 1974.
- ¹² Pease AA. Biochemical identification of the enterobacteriaceae using a multipoint inoculation system. *Med Lab Sci* 1983;**40**:349–53.
- ¹³ Brown DJF, Warner M, Taylor CED, Warren RE. Automated detection of micro-organisms in blood cultures by means of the Malthus microbiological growth analyser. *J Clin Pathol* 1984;**37**:65–9.

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